

genesis, genetic disease, and cancer. Hedgehog acts as a morphogen to specify distinctive cell fates using different concentration thresholds but our knowledge of how the concentration gradient is interpreted into the activity gradient is incomplete. The membrane protein Growth Arrest Specific Gene 1 (GAS1) was thought to be a negative regulator of the Hedgehog concentration gradient. Here, we report unexpected genetic evidence that *Gas1* positively regulates Hedgehog signaling in multiple developmental contexts, particularly at regions where Hedgehog acts at low concentration. Using a combination of *in vitro* cell culture and *in ovo* electroporation assays, we demonstrate that GAS1 acts cooperatively with Patched1 for Hedgehog binding and enhances signaling activity in a cell-autonomous manner. Our data support a model in which GAS1 helps transform the diffusible Hedgehog protein gradient into the observed activity gradient. We propose that *Gas1* is an evolutionarily novel, vertebrate-specific Hedgehog pathway regulator and that it may also contribute to the disease states caused by misregulated Hedgehog signaling.

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Program/Abstract # 109

Genetic analysis of FGF signaling in axis extension and somitogenesis

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Various models have assigned FGF signaling, particularly through *Fgf8*, a pivotal role in somitogenesis/mesodermal segmentation. However, our recently published work demonstrates that *Fgf8* is not required for somite development in the mouse, suggesting that any potential role *Fgf8* plays in this process would be redundant with other members of the FGF family. Therefore we have analyzed mice with primitive streak-specific inactivation (via conditional gene control using the TCre line) of *Fgf8* concurrently with deletions in each of the other *Fgf* genes expressed in the primitive streak or presomitic mesoderm (*Fgf3*, 4, 5, 17 and 18). Inactivation of both *Fgf4* and 8 causes an axis truncation and only the first six–eight somites form. Preliminary evidence suggests that axis truncation occurs because of a depletion of a primitive streak stem cell progenitor population. Mutant TCre/*Fgf4*/*Fgf8* somites are smaller and exhibit patterning defects. Molecular analysis indicates that *Fgf4/8* is required for normal expression of somitic clock genes, which are required for proper segmentation. To explore potential interactions between different signaling pathways that regulate somite development, we have performed genetic experiments that complex our TCre/*Fgf4*/*Fgf8* mutations with mutant components in the WNT and retinoic acid signaling pathways. These experiments reveal aspects of crosstalk between these

different signaling pathways during axis extension and somitogenesis.

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Program/Abstract # 110

Wnt signaling and ventral dermis development in the mouse embryo

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The skin consists of two layers: an outer layer called the epidermis and an inner layer, the dermis. The dermis induces and supports the epidermal appendages of the skin. Loss of dermis from birth defects, deep wounds and burns compromises the function of the skin. Dermis originates from the somites, lateral plate mesoderm, and cranial crest cells in the chick embryo. We use genetic tools to identify the origin and inductive signals for ventral dermis in a mammalian model, the mouse embryo. First, we lineage mapped the lateral plate mesoderm (LPM) with different genetic tools. In our analysis at the forelimb level, tagged cells were first found in the flank mesenchyme, and later in the entire ventral trunk dermis, limb mesoderm, body wall muscle, sternum, but not in the epidermis. Wnt signaling is required in dorsal dermal induction and development in the chick and mouse embryo. We have used temporally restricted genetic tools to create a tissue-specific conditional mutant of beta-catenin, the central transducer of the Wnt signal in the ventrum. In the absence of Wnt signal transduction in the LPM, the ventral dermis fails to develop. Ongoing studies focus on identifying the mechanism of Wnt signaling/beta-catenin function in ventral dermis induction and development. Our results to date are the first genetic studies to demonstrate that the ventral dermis in the mouse embryo originates from the LPM and Wnt signaling is critical for normal ventral dermis development.

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Program/Abstract # 111

The role of Notch signaling in mouse lens development

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The *Notch* signaling pathway regulates proliferation, cell shape changes, differentiation and stem cell maintenance in many tissues. Recently, we established a requirement for the *Notch* effector *Hes1* during mouse lens cell proliferation. Interestingly, *Notch1*^{-/-}, *Notch2*^{-/-} and *Jagged1*^{+/-} mutant

mice have prenatal eye phenotypes (1–3), and Alagille Syndrome patients with *Jagged1* mutations exhibit anterior eye deformities (4). But, very little is known about the roles of *Notch* signaling in eye induction, growth or morphogenesis. We found that the ligand *Jagged1*, and two receptors, *Notch1* and *Notch2*, are expressed during lens placode invagination, vesicle morphogenesis and fiber cell differentiation. To determine the requirements for *Notch* signaling in the lens, we are conditionally deleting *Notch* pathway genes in this tissue. First we examined loss of the vertebrate *Su(H)* orthologue, *RBP-JK1*, which complexes with the *Notch* intracellular domain to transduce canonical signaling via *Hes1* transcriptional activation. When *RBP-JK1* was removed during embryonic lens formation, we observed reciprocal changes in lens progenitor cell proliferation versus fiber cell differentiation, consistent with reduced proliferation in *Hes1* mutants. Our model for *Notch* regulation of lens proliferation and differentiation will be presented. 1. Development 124: 1139–1148 (1997) 2. Development 128: 491–502 (2001) 3. Human Molecular Genetics 8: 723–730 (1999) 4. Nature Genetics 16: 235–242 (1997).

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Program/Abstract # 112

The Yin and Yang of Notch signalling; trans-activation and cis-inhibition fine-tune Notch signalling

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The Notch receptor is *trans*-activated by single pass transmembrane ligands present on the surface of neighbouring cells. In mammals Dll1, Dll4, Jag1 and Jag2 can activate the Notch1 receptor in trans, and inhibit Notch1 signalling when expressed in the same cell as the receptor. It is considered that Notch signalling must be tightly regulated, and we propose that this is achieved through the interplay of *trans*-activation and *cis*-inhibition. Using an in vitro co-culture system that recapitulates Notch signalling, we show that Dll3 (the most divergent ligand of Notch) cannot *trans*-activate Notch1 but can *cis*-inhibit signalling. In addition we show that Dll3, unlike the other ligands, localises to the Golgi apparatus in preference to the cell surface. Furthermore, mutant forms of Dll3 that cause the congenital vertebral malsegmentation disorder spondylocostal dysostosis (SCD) are mislocalised. In Dll3 null mouse embryos and individuals with SCD, somite formation is abnormal resulting in vertebral anomalies. Therefore, we examined Notch1 signalling in the presomitic mesoderm (PSM) of Dll3-null embryos by immunohistochemistry. Comparison of these cells in the PSM with those that express Dll3 demonstrates that in Dll3 null mouse embryos, the domain in which Notch1 is activated is expanded indicating that Dll3 is required to restrict Notch1

signalling in the PSM. This is consistent with an inhibitory role for Dll3 in Notch1 signalling.

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Program/Abstract # 113

The role of endocytosis in activin signalling during mesoderm induction in *Xenopus*

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Work from several laboratories has shown that endocytosis plays a critical role in modulating morphogen signalling, transmission and response during embryonic development. I am investigating to what extent endocytic trafficking influences mesoderm induction by Xnr2 and activin in the *Xenopus* embryo, both in terms of the signalling ranges of these molecules and in terms of the ways that cells respond to them. I have used a dominant-negative form of dynamin and RN-tre to inhibit endocytosis, and observed signalling in real time using GFP-tagged forms of these TGF- β family members, as well as Alexa 488-labelled activin. My data show that inhibition of endocytosis by RN-tre injection decreases morphogen signalling ranges, and up-regulation of endocytosis by over-expressing Rab5 increases it. Use of quantitative RT-PCR indicates that these endocytic reagents have little effect on the ability of the cells to respond to activin. I am now further investigating to what extent endocytosis affects signal production and signal transmission.

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Program/Abstract # 114

The role of *Endothelin-1/Endothelin Receptor A* signaling in neural crest specification and cell survival

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The neural crest (NC) is a multipotent cell population that gives rise to different tissues after extensive migration along the vertebrate embryo. In the frog *Xenopus laevis*, the NC is specified in the ectoderm from late gastrula stage by interactions between different signals emanated from the epidermis, neural plate and mesoderm. It has been proposed that neural crest induction is a multi step process, but so far these different steps are not completely understood. In this work, we show for the first time the existence of a NC maintenance step which occurs after the initial induction of NC, and that is dependent on Edn1 signal released from the mesoderm underlying the neural crest at the neurula stage. We have cloned *Xenopus Preproendothelin-1* and *ECE-1* homologues, and the analysis of the expression